

JCM01223-17R1

Supplementary Figure 1. Design of primers and probe from *ITS2*

gene. Multiple alignments of *ITS2* gene comprising all phylogenetic clades of *C. auris* and other closely related yeast species resulted in a combination of sequences used for the design of primers and probe for *C. auris*. The GenBank numbers for strains used for alignments are AB375772 *Candida auris* East Asia clade; KC692039 *Candida auris* South Asia clade; KJ126758 *Candida auris* Africa clade; KT305985 *Candida auris* South America clade; KX810325 *Candida auris* East Asia clade; KX870921 *Candida auris* South Asia clade; KX870919 *Candida duobushaemulonii*; KX870918 *Candida haemulonii*; JX459678 *Candida pseudohaemulonii*; AF246989 *Candida krusei*; KU729100 *Candida lusitaniae*.

Supplementary Figure 2. Specificity assessment of *C. auris* real-time PCR assay in actual surveillance samples negative for *C. auris* DNA.

The surveillance samples harboring other organisms (recovered in culture) or harboring no organisms (no growth in culture) did not cross react in *C. auris* real-time PCR assay further confirming high specificity of the real-time PCR assay. The organisms recovered are listed below the pie chart for swabs (A) and sponges (B).

Supplementary table 1. Inter-assay reproducibility of the real-time PCR assay

Level	Yeast CFU/ 50 µl	Yeast CFU/ PCR Rxn	Day 1			Day 2			Day 3			Mean Ct ± SD	% CV
			Ct 1	Ct 2	Ct 3	Ct 1	Ct 2	Ct 3	Ct 1	Ct 2	Ct 3		
High	10^5	10^4	21.99	21.62	21.61	22.56	22.59	22.83	22.02	22.14	22.15	22.17 ± 0.42	1.90
Moderate	10^3	10^2	30.14	30.15	30.15	30.84	30.96	30.40	31.66	30.77	31.01	30.68 ± 0.51	1.67
Low	10^2	10^1	33.30	33.65	33.28	33.66	33.76	33.80	33.91	33.86	34.30	33.72 ± 0.31	0.92

Supplementary table 2. Intra-assay reproducibility of the real-time PCR assay

Level	Yeast CFU/50 µL	Yeast CFU/Rxn	Ct 1	Ct 2	Ct 3	Mean Ct ± SD	% CV
High	10^5	10^4	21.99	21.62	21.61	21.74 ± 0.22	0.99
Moderate	10^3	10^2	30.14	30.15	30.15	30.15 ± 0.01	0.02
Low	10^2	10^1	33.30	33.65	33.28	33.41 ± 0.21	0.62

Supplementary Table 3. *C. auris* real-time PCR assay specificity

Isolate No.	Organism	Source	<i>C. auris</i> Mean Ct
-	NTC	-	0
<i>Candida auris</i>			
M5658	<i>C. auris</i> (South Asia, Clade I)	New York, USA	18.78
M5587	<i>C. auris</i> (South Asia, Clade I)	New York, USA	18.46
M5695	<i>C. auris</i> (South Asia, Clade I)	New York, USA	18.39
M5676	<i>C. auris</i> (South Asia, Clade I)	New York, USA	18.72
AR0382	<i>C. auris</i> (South Asia, Clade I)	FDA-CDC AR Bank	18.39
AR0387	<i>C. auris</i> (South Asia, Clade I)	FDA-CDC AR Bank	18.81
AR0388	<i>C. auris</i> (South Asia, Clade I)	FDA-CDC AR Bank	17.85
AR0389	<i>C. auris</i> (South Asia, Clade I)	FDA-CDC AR Bank	18.14
AR0390	<i>C. auris</i> (South Asia, Clade I)	FDA-CDC AR	18.36
M5692	<i>C. auris</i> (East Asia, Clade II)	New York, USA	19.06
M5657	<i>C. auris</i> (East Asia, Clade II)	New York, USA	18.63
M5691	<i>C. auris</i> (East Asia, Clade II)	New York, USA	18.80
AR0381	<i>C. auris</i> (East Asia, Clade II)	FDA-CDC AR Bank	17.68
AR0383	<i>C. auris</i> (Africa, Clade III)	FDA-CDC AR Bank	18.63
AR0384	<i>C. auris</i> (Africa, Clade III)	FDA-CDC AR Bank	19.16
AR0385	<i>C. auris</i> (South America, Clade IV)	FDA-CDC AR Bank	18.79
AR0386	<i>C. auris</i> (South America, Clade IV)	FDA-CDC AR Bank	18.87
Other <i>Candida</i> spp.			
M175	<i>Candida albicans</i>	MCC, NYSDOH	0
M642	<i>C. blankii</i>	MCC, NYSDOH	0
M3193	<i>C. bracarensis</i>	MCC, NYSDOH	0
M5164	<i>C. ciferrii</i>	MCC, NYSDOH	0
M4363	<i>C. colliculosa</i>	MCC, NYSDOH	0
M314	<i>C. dubliniensis</i>	MCC, NYSDOH	0
M3051	<i>C. duobushaemulonii</i>	MCC, NYSDOH	0
M4625	<i>C. duobushaemulonii</i>	MCC, NYSDOH	0
M5690	<i>C. duobushaemulonii</i>	MCC, NYSDOH	0
M4831	<i>C. fabianii</i>	MCC, NYSDOH	0
M4572	<i>C. famata</i>	MCC, NYSDOH	0
M208	<i>C. glabrata</i>	MCC, NYSDOH	0
M446	<i>C. guilliermondii</i>	MCC, NYSDOH	0
M5659	<i>C. haemulonii</i>	MCC, NYSDOH	0
M1081	<i>C. inconspicua</i>	MCC, NYSDOH	0
M4187	<i>C. intermedia</i>	MCC, NYSDOH	0
M3968	<i>C. kefyr</i>	MCC, NYSDOH	0
M3221	<i>C. krusei</i>	MCC, NYSDOH	0
M2840	<i>C. lipolytica</i>	MCC, NYSDOH	0
M240	<i>C. lusitaniae</i>	MCC, NYSDOH	0
M4430	<i>C. metapsilosis</i>	MCC, NYSDOH	0
M2717	<i>C. norvegensis</i>	MCC, NYSDOH	0

M4903	<i>C. orthopsilosis</i>	MCC, NYSDOH	0
M130	<i>C. parapsilosis</i>	MCC, NYSDOH	0
M4221	<i>C. propengiesseri</i>	MCC, NYSDOH	0
M3491	<i>C. quercitrusa</i>	MCC, NYSDOH	0
M3455	<i>C. rugosa</i>	MCC, NYSDOH	0
M3919	<i>C. sorbovorans</i>	MCC, NYSDOH	0
M15725	<i>C. zeylanoides</i>	MCC, NYSDOH	0
M1645	<i>Cryptococcus gattii</i> (NIH 444)	MCC, NYSDOH	0
M4758	<i>C. laurentii</i>	MCC, NYSDOH	0
M4909	<i>C. neoformans</i>	MCC, NYSDOH	0
M135	<i>C. neoformans</i> var. <i>grubii</i> (H99)	MCC, NYSDOH	0
M2383	<i>C. neoformans</i> var. <i>neoformans</i> (NIH12)	MCC, NYSDOH	0
M277	<i>Geotrichum capitatum</i>	MCC, NYSDOH	0
M133	<i>Kluyveromyces maxianus</i>	MCC, NYSDOH	0
M4920	<i>Rhodotorula mucilaginosa</i>	MCC, NYSDOH	0
M275	<i>R. rubrum</i>	MCC, NYSDOH	0
M78	<i>Saccharomyces cerevisiae</i>	MCC, NYSDOH	0
M205	<i>Trichosporon asahii</i>	MCC, NYSDOH	0
Mold species			
M5499	<i>Aspergillus flavus</i>	MCC, NYSDOH	0
M5414	<i>A. fumigatus</i>	MCC, NYSDOH	0
M3319	<i>A. glaucus</i>	MCC, NYSDOH	0
M4737	<i>A. nidulans</i>	MCC, NYSDOH	0
M4355	<i>A. oryzae</i>	MCC, NYSDOH	0
M5370	<i>A. sydowii</i>	MCC, NYSDOH	0
M5321	<i>A. terreus</i>	MCC, NYSDOH	0
M4819	<i>A. versicolor</i>	MCC, NYSDOH	0
M4293	<i>Mucor circinelloides</i>	MCC, NYSDOH	0
Bacterial spp.			
DSM7312	<i>Aeromonas enteropelogenes</i>	BCC, NYSDOH	0
CDCBC3133	<i>Bacillus cereus</i>	BCC, NYSDOH	0
ATCC6051	<i>B. subtilis</i>	BCC, NYSDOH	0
ATCC33559	<i>Campylombacter coli</i>	BCC, NYSDOH	0
ATCC33291	<i>C. jejuni</i>	BCC, NYSDOH	0
NYS-08-6620	<i>Clostridium difficile</i>	BCC, NYSDOH	0
ATCC51434	<i>Escherichia coli</i> 0157: H7	BCC, NYSDOH	0
ATCC BAA-181	<i>E. coli</i> STEC	BCC, NYSDOH	0
ATCC19114	<i>Listeria monocytogenes</i>	BCC, NYSDOH	0
ATCC13076	<i>Salmonella enteritidis</i>	BCC, NYSDOH	0
ATCC14028	<i>S. typhimurium</i>	BCC, NYSDOH	0
ATCC12022	<i>Shigella flexneri</i>	BCC, NYSDOH	0
ATCC14458	<i>Staphylococcus aureus</i>	BCC, NYSDOH	0
ATCC17749	<i>Vibro alginolyticus</i>	BCC, NYSDOH	0
WC-33114	<i>Yersinia enterocolitica</i>	BCC, NYSDOH	0
Parasite spp.			
16-19165	<i>Babesia microti</i>	Parasitology, NYSDOH	0

16-19325	<i>Plasmodium falciparum</i>	Parasitology, NYSDOH	0
15-62007	<i>P. malariae</i>	Parasitology, NYSDOH	0
16-8664	<i>P. ovale</i>	Parasitology, NYSDOH	0
16-14201	<i>P. vivax</i>	Parasitology, NYSDOH	0
Viral spp.			
S-1	<i>Lentivirus HIV-2</i>	Virology, NYSDOH	0
S-2	<i>Orthohepadnavirus hepatitis B virus</i>	Virology, NYSDOH	0

MCC, Mycology Culture Collection; BCC, Bacterial Culture Collection;

NYSDOH, New York State Department of Health; FDA-CDC AR, Food & Drug Administration-Center for Diseases Control Antibiotic Resistant; ATCC, American Type Culture Collection

Supplementary Table 4. *Candida auris* detection in the spiked surveillance samples (swabs and sponges) by real-time PCR assay

Swabs	Level	CFU/50 µl	Yeast CFU/Rxn)	No. Samples Tested	Mean Ct ± SD	% CV
<i>C. auris</i>	High	10 ⁵	10 ⁴	10	20.51 ± 0.47	2.27
	Moderate	10 ³	10 ²	10	30.07 ± 0.35	1.15
	Low	10 ²	10 ¹	10	33.52 ± 0.61	1.81
<i>Candida</i> spp.	Moderate	10 ³	10 ²	10	0.0 (Undet)	0
Sponges						
<i>C. auris</i>	High	10 ⁵	10 ⁴	10	22.08 ± 1.26	5.71
	Moderate	10 ³	10 ²	10	31.56 ± 0.60	1.91
	Low	10 ²	10 ¹	10	35.51 ± 1.25	3.52
<i>Candida</i> spp.	Moderate	10 ³	10 ²	10	0.0 (Undet)	0

Supplementary Fig. 1. Multiple alignment of *ITS2* gene of *C. auris* and other closely related *Candida* species.

	1	10	20	30	40	50	60	70	80	90	100	110	120	130	140
1. V2424 (CAURF)	CAGA	--	CGTGAATCATCGAACATCT												
2. V2425 (CAURP)															
3. V2426 (CAURR)															
4. AB375772	ACTTGCGA	--	CGTGAATCATCGAACATCTTGAACGCACATTGCGCCTGGGTATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACAA	TCTCGCGGTGGCGTTGCATTCA	CACAA	AAATTACAGCTTGACGAAA									
5. KC692039	ACTTGCGA	--	CGTGAATCATCGAACATCTTGAACGCACATTGCGCCTGGGTATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACAA	TCTCGCGGTGGCGTTGCATTCA	CACAA	AAATTACAGCTTGACGAAA									
6. KJ126758	ACTTGCGA	--	CGTGAATCATCGAACATCTTGAACGCACATTGCGCCTGGGTATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACAA	TCTCGCGGTGGCGTTGCATTCA	CACAA	AAATTACAGCTTGACGAAA									
7. KT305985	ACTTGCGA	--	CGTGAATCATCGAACATCTTGAACGCACATTGCGCCTGGGTATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACAA	TCTCGCGGTGGCGTTGCATTCA	CACAA	AAATTACAGCTTGACGAAA									
8. KX810325	ACTTGCGA	--	CGTGAATCATCGAACATCTTGAACGCACATTGCGCCTGGGTATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACAA	TCTCGCGGTGGCGTTGCATTCA	CACAA	AAATTACAGCTTGACGAAA									
9. KX870921	ACTTGCGA	--	CGTGAATCATCGAACATCTTGAACGCACATTGCGCCTGGGTATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACAA	TCTCGCGGTGGCGTTGCATTCA	CACAA	AAATTACAGCTTGACGAAA									
10. KX870919	ACTTGCGA	--	CGTGAATCATCGAACATCTTGAACGCACATTGCGCCTGGGTATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACAA	TCTCGCGGTGGCGTTGCATTCA	CACAA	AAATTACAGCTTGACGAAA									
11. KX870918	ACTTGCGA	--	CGTGAATCATCGAACATCTTGAACGCACATTGCGCCTGGGTATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACAA	TCTCGCGGTGGCGTTGCATTCA	CACAA	AAATTACAGCTTGACGAAA									
12. JX459678	ACTTGCGA	--	CGTGAATCATCGAACATCTTGAACGCACATTGCGCCTGGGTATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACAA	TCTCGCGGTGGCGTTGCATTCA	CACAA	AAATTACAGCTTGACGAAA									
13. AF246989	AATTGCGAGCCAT	CGTGAATCATCGA	GTTGAAACGCACATTGCGCC	CGGGG	GGCATGCCCTGGGATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACCGG	AGGTGCTTGCATCCGTA	AAATAACATA	TCCGACGGAAGGTG	CGTACGG	AAAGAGGGT	CG	GACGTGAAAGAGGGT	CG		
14. KU729100	ACTTGCGA	--	CGTGAATCATCGAACATCTTGAACGCACATTGCGCCTCGAGGATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACCG	GGCATGCCCTGGGATTCCCCAAGGCATGCCCTGGGTGAGCGTGATGCTTCACCGG	ACGCTTGCATCCGTA	AAATAACATA	TCCGACGGAAGGTG	CGTACGG	AAAGAGGGT	CG	GACGTGAAAGAGGGT	CG			

Supplementary Figure 2. Specificity assessment of *C. auris* real-time PCR assay in actual surveillance samples negative for *C. auris* DNA.



